Proteomic strategies for analysis of cerebrospinal fluid in neurodegenerative disorders

AKADEMISK AVHANDLING

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av

Sara Hansson

Fakultetsopponent: Professor Jonas Bergquist, Institutionen för fysikalisk och analytisk kemi, Uppsala universitet

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Avhandlingen baseras på följande delarbeten:

I. Validation of a prefractionation method followed by two-dimensional electrophoresis - Applied to cerebrospinal fluid proteins from frontotemporal dementia patients.
Hansson SF, Puchades M, Blennow K, Sjogren M, Davidsson P. *Proteome Sci. 2004 Nov 18;2(1):7.*

II. Proteomic studies of potential cerebrospinal fluid protein markers for Alzheimer's disease.
Puchades M*, **Hansson SF***, Nilsson CL, Andreasen N, Blennow K, Davidsson P. *Brain Res Mol Brain Res. 2003 Oct 21;118(1-2):140-6.*

III. Reduced levels of amyloid-β-binding proteins in cerebrospinal fluid from Alzheimer's disease patients
Hansson SF, Andreasson U, Wall M, Skoog I, Andreasen N, Wallin A, Zetterberg H, Blennow K. *Submitted.*

IV. Cystatin C in cerebrospinal fluid and multiple sclerosis.
Hansson SF, Hviid Simonsen A, Zetterberg H, Andersen O, Haghighi S, Fagerberg I, Andreasson U, Westman-Brinkmalm A, Wallin A, Ruetschi U, Blennow K. *Ann Neurol. 2007 Aug;62(2):193-6.*

V. Characterization of tau in cerebrospinal fluid using mass Spectrometry.
Portelius E*, **Hansson SF***, Tran AJ, Zetterberg H, Grognet P, Vanmechelen E, Brinkmalm G, Westman-Brinkmalm A, Nordhoff E, Blennow K and Gobom J. *J Proteome Res, in press 2008.*

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Sara Hansson
Institute of Neuroscience and Physiology, University of Gothenburg, Sweden

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ABSTRACT

There is a great need for biomarkers to diagnose neurodegenerative disorders, such as the cognitive disorders Alzheimer's disease (AD) and frontotemporal dementia (FTD). Cerebrospinal fluid (CSF) is in contact with the extracellular fluid of the brain and is consequently a valuable medium for identifying biomarkers for neurological disorders. Biomarkers can be used for early identification of disease, to facilitate homogenous classification, and to extend our basic knowledge of disease pathogenesis. Proteomics, an approach for biomarker discovery, generally combines various separation techniques with mass spectrometry (MS) and bioinformatics to identify and characterize proteins, reflecting a defined state at a specific time point. The aim of this thesis was to develop and evaluate proteomic strategies for analysis of CSF proteins to reveal disease mechanisms and identify potential biomarkers to distinguish AD from FTD.

Two approaches to improve the detection of CSF proteins by two-dimensional gel electrophoresis (2-DGE) were used. First, to enrich the proteins, CSF was prefractionated using liquid phase isoelectric focusing followed by 2-DGE profiling. Secondly, zoom 2D gels increased protein separation directly in the gels. These studies showed that in the CSF proteome of AD and FTD patients several proteins were differentially expressed, suggesting that different mechanisms are involved in the pathogenesis of these disorders.

To validate some of the findings from the 2-DGE studies, β-trace, transthyretin (TTR), α-1-antitrypsin and cystatin C (CysC) were quantified in CSF. The concentrations of all these proteins, previously shown to bind amyloid-beta (Aβ) peptides, were reduced in AD CSF, while only CysC and β-trace were reduced in FTD. Furthermore, we found a strong positive correlation between β-trace, TTR and CysC, and levels of Aβ peptides specifically in the AD group, suggesting that a lack of proteins binding to Aβ peptides in AD CSF might cause increased extracellular Aβ aggregation, a major pathological hallmark in the AD brain.

Additionally, we showed that incorrect storage conditions can influence the isoform levels of some CSF proteins. Thus, standardization of CSF sample handling is important in avoiding ambiguous results. Furthermore, very low-abundant neuron specific tau protein isoforms, were for the first time characterized in CSF using a targeted immunoprecipitation-MS approach, opening up new possibilities for further differentiation of tauopathies, including AD and FTD.

Key words: Alzheimer's disease, cerebrospinal fluid, frontotemporal dementia, neurodegeneration, proteomics, mass spectrometry, prefractionation, protein identification, quantification

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